IRON ASSIMILATION BY MARINE DIATOMS[1]

EDWARD D. GOLDBERG

Scripps Institution of Oceanography, La Jolla, California

The relationship of marine productivity to the availability of iron has been discussed by Gran (1933), who suggested that organically combined iron introduced into coastal waters from terrestrial run-off is responsible for phytoplankton abundances. He substantiated his argument by demonstrating that the growth of the neritic diatom *Skeletonema costatum* was stimulated by soil extract containing 0.2 micromole of iron per liter, although the iron bound into oxyhemoglobin molecules had no effect. Hopkins (1930) has indicated that ionic iron is necessary for the growth of *Chlorella,* and Rodhe (1948) defines the available iron for utilization in culture by fresh water algae as that which has been stabilized by combination with citrate.

Harvey (1937a) has demonstrated that diatoms are able to assimilate ferric hydroxide or ferric phosphate in colloidal or particulate form and to utilize it in their growth. He has further shown, using the calculated saturation concentrations of ferric and ferrous ions in marine waters, that diatoms accumulate 10,000 times more iron than could possibly be obtained from diffusion of iron ions from the environmental waters. His treatment considered a stationary spherical living cell with respect to the environment, whereas a falling diatom would encounter and take up somewhat more iron.

Continuing an investigation of the minimal quantities of mineral nutrients needed for optimal growth of the marine diatom *Asterionella japonica* (Goldberg, Walker and Whisenand, 1951), an assessment of iron uptake presents the following questions. What is the minimal content of iron per cell needed for further division? What constitutes available iron? Finally, inasmuch as the concentration of iron in marine waters has been found to be highly variable (Cooper, 1948), can iron content be one of many possible parameters in the nutrient index of the productivity equation of Riley, Stommel and Bumpus (1949)?

CULTURE TECHNIQUES

The culture techniques used in the following experiments, unless otherwise indicated, have been described previously in the work on phosphate uptake (Goldberg, Walker and Whisenand, 1951). To maintain symmetry with respect to these investigations the same culture of *Asterionella japonica,* which was isolated from the plankton samples in the winter of 1950, was used. The initial phosphate level was adjusted to 2.5 to 3.0 micromoles of phosphate per liter.

---

[1] Contribution of the Scripps Institution of Oceanography, New Series No. 572.

IRON ASSAY

The iron determinations were made either by the colorimetric method of Buch (1942) or through the use of radioactive iron. In the former procedure the extinction of the alpha-alpha' bipyridyl complex of iron was measured at 522 $m\mu$ in 100 mm. cells in a Beckman Model DU Spectrophotometer. The method determines both ferric and ferrous iron, but only a portion of the organically bound or particulate iron.

Iron[2] containing the radioactive isotopes Fe^{55}, which decays by K capture, and Fe^{59}, a beta and gamma emitter, was employed as a tracer for this element. The radio iron was plated from aliquots of sea water samples onto 10 $cm.^2$ copper planchets following the procedure of Ross and Chapin (1942). As assayed under an end-window Geiger-Muller tube with a window thickness of 1.8 $mg./cm.^2$, the specific activity was 2500 counts per minute per micromole.

IRON PARTITION IN CULTURE BOTTLES

In the first experiments with radioactive iron it was found that 80 to 90 per cent of the iron added initially as the ferric citrate complex was adsorbed within one day to the walls of the culture bottle. To eliminate this adsorption, the culture bottles were coated with "Desicote," a polymer of silicon (manufactured by Beckman Instruments, Inc., South Pasadena, California). The coated bottles had no adverse effect upon diatom growth, as was evident from control runs in both treated and untreated bottles. The bottles retained their coating for periods up to one month. At the end of an experiment the bottles were washed with a detergent and thoroughly rinsed with distilled water.

Radioactive ferric citrate, freshly prepared, was introduced into sea water of pH 8.00 to give an iron concentration of 2.2 micromoles per liter. Aliquots of 10 ml. of the solution were centrifuged for thirty minutes and the supernatant liquid assayed radiometrically for iron. The results are listed in Table I. It is observed that hydrolysis of iron citrate occurs to a considerable extent in a period of four days.

As is noted in the table, the decomposition was noticeably accelerated both by light and by stirring. The light effect is probably similar to that reported by Peltz and Lynn (1938) who found that iron citrate complex was partially decomposed in sunlight to CO_2 and a soluble ferrous ion. The ferrous ion formed in this way can readily be oxidized to give the ferric hydroxide. The more pronounced effect of stirring upon the hydrolysis is undoubtedly due to the formation of large particulate masses by collisions of smaller iron hydroxide particles, and to more rapid oxidation of any ferrous ion formed.

As pointed out by Harvey (1945), iron ascorbate has less tendency to become hydrolyzed in saline media, if the solution is freshly prepared. On standing, stock solutions of iron ascorbate, originally tinted but a very light green, assume deep brown colorations. It was found that ferric ascorbate was not decomposed for periods up to one week, but after longer periods the formation of centrifugable iron hydroxide followed the course of iron citrate solutions. Iodoform tests on both the

---

[2] The radioactive iron metal used in this investigation was supplied by the Oak Ridge National Laboratory on allocation from the Isotopes Division, U. S. Atomic Energy Commission.

TABLE I

The hydrolysis of iron complexes in sea water solutions

Complex of ferric ion	Time elapsed since addition of iron complex (days)	% of original iron remaining in supernate	Remarks
Citrate	0	100	Light, stirred
	1	64	
	2	29	
	3	20	
	4	11	
	6	6	
Citrate	4	50	Dark, unstirred
	4	10	Light, stirred
	4	31	Light, unstirred
Ethylene diamine tetra-acetate	0	100	Light, stirred
	1	88	
	3	80	
	6	15	

ferric citrate and ferric ascorbate solutions were positive within two weeks after preparation.

Ethylenediamine tetra-acetic acid chelates with trivalent iron and this complex was studied as a potential iron source. However, after six days 85 per cent of the iron had hydrolyzed at a pH of 8.25. These results are consistent with the experiments of Jacobson (1951) who found instability of the complex in basic aqueous solutions.

It should be noted that in all cases involving the complexing of iron, the iron and complexing agent were mixed in a 1 : 2 molar proportion. The iron concentration was 2.2 micromoles/liter.

UPTAKE OF IRON BY ASTERIONELLA JAPONICA

In order to establish a minimal cell content of iron necessary for further growth, varying amounts of iron in the form of iron citrate were added to the culture bottles with an initial diatom inoculum of 300–400 cells/ml. The culture medium was assayed for iron colorimetrically before the addition of iron citrate. If the nutrient water showed no iron content, it was used in the growth experiments. It was normally found that inshore surface sea water filtered through a Whatman No. 42 paper was free of iron within the limit of the analytical method (0.1 micromole per liter).

The results of a typical experiment are given in Table II. No growth was observed until the initial iron concentration exceeded 0.8 micromole per liter. Both growth and final population of the cells could be controlled by the initial iron concentration. The iron per diatom, when leveling-off of the exponential growth occurred, averaged 1.0×10^{-7} micromoles per cell. We have assumed that essentially all the iron was removed by the biomass. Since the maximum cell densities were not reached until 6 or 8 days after inoculation, the greater part of the iron available to the diatoms was in particulate form.

TABLE II

Iron uptake by Asterionella japonica

Culture bottle	1	2	3	4	5	6
Initial iron in micromoles/liter	0.8	1.6	2.2	2.5	3.4	3.4
Final diatom population cells/ml.	0	14,700	21,700	18,200	41,900	41,100
Final iron content of diatoms micromoles/cell × 10^7	0	1.1	1.0	1.4	0.81	0.82

Initial diatom inoculum: 300 cells/ml.
Initial phosphate: 2.62 micromoles/liter.

Humic acid has been reported as a complexing agent which makes iron more readily available to various plants and micro-organisms. Harvey (1937b) found increased growth with an artificial iron humate, but the effect was less than that of the iron citrate complex. Although the chemical formulae for the various iron complexes that are present in both natural and synthetic preparations are undetermined, it was desirable to ascertain whether iron humate might be a suitable nutrient source for *Asterionella japonica.* The synthetic humic acid and iron humate solutions were prepared according to the method of Burk, Lineweaver and Horner (1932). The iron content of the iron humate solutions was determined colorimetrically after a wet digestion with 3:1 nitric: perchloric acid mixture. The iron humate does not give centrifugable products from sea water solutions when prepared in this fashion.

The results of the humate experiments are given in Table III. Iron citrate was added to control cultures. The initial phosphate level was 2.7 micromoles per liter and the diatom inoculum was 750 cells/ml. Humate as the source of iron did not promote growth. However, the excess humic acid constituents did not deter diatom growth, as is seen in the results of bottle 5. We may therefore conclude that this form of iron humate is not available for metabolic utilization by *Asterionella japonica.*

Freshly prepared radioactive iron ascorbate was added to the culture bottles to give an initial concentration of 1.45 micromoles per liter. Three moles of ascorbate were present for each mole of iron. Exponential diatom growth was observed up to 30,000 cells per ml.; however, the cells appeared misshapen and unhealthy. No detectable iron was removed from the solution by radioactive assay of

TABLE III

Diatom growth experiments with ferric humate as the source of iron

Culture flask	1	2	3	4	5	6
Iron form	Humate	Citrate	Humate	Citrate	Citrate + Humic acid	Humate
Initial iron concentration micromoles/liter	12.5	14	12.5	14	14	6.3
Final concentration diatoms cells/ml.	0	81,000	0	62,000	70,000	0

the supernatant solution. To confirm this finding the diatoms were filtered, digested in the 3:1 nitric: perchloric acid mixture and the iron activity was determined radiometrically. The cell content of the accumulated radio-iron averaged 1.9×10^{-9} micromoles per cell. This is about 2% of the minimal iron content as determined from the uptake experiments previously described. The initial amount of particulate iron in the culture flasks was 1.8 micromoles per liter added as ferric chloride solution.

One final experiment was undertaken to confirm the observation that in the absence of complexed ferric iron and in the presence of particulate iron, diatom growth could proceed. Cells from a growing sub-culture were placed in a dialyzing sac which held 50 ml. of nutrient solution such that there was an initial diatom population of 800 cells/ml. The sac was held in place by a rubber stopper in a 250 ml. volumetric cylinder which contained 210 ml. of solution. Freshly prepared iron citrate was introduced into either the dialyzing sac or the cylinder to give an iron concentration of 2.2 micromoles per liter. In the former case the ionic complexed iron penetrated the membrane to give a concentration of 0.01 micromole per liter after 2 days, *i.e.,* 20% of the iron diffused into the outer cylinder. Here normal exponential growth occurred, whereas when the diatoms could receive only the complexed iron as in the latter case, no population increases were noted.

DISCUSSION

From the above experiments it is evident that the organic iron complexes used were not available as a growth nutrient to marine diatoms, whereas particulate and/or colloidal forms of iron are utilized in their metabolism.

Cooper (1935) found that the ratios of iron to phosphorus, in plankton samples consisting almost entirely of diatoms, were 4.2 and 4.4 in two separate determinations. From the experiments of phosphate uptake by *Asterionella japonica*, 5×10^{-8} micromoles of phosphate per cell were necessary for division. Combining this with the minimum iron content of 10×10^{-8} micromoles/cell, we calculate for our cultural populations

$$\mathrm{Fe/P} = \frac{55.85 \times 10 \times 10^{-8}}{30.98 \times 5 \times 10^{-8}} = 3.6$$

The striking agreement between these values suggests that this method of study of cultural diatom populations represents a fair simulation of ocean growth.

Cooper (1948) attributes the extreme variability in iron content in the upper layers of marine waters as due to the random distribution of particles of a large size (*i.e.*, 160 μ), as large as some sand grains. It would be of interest to know what size of iron hydroxide particle might be necessary for diatom division. We may assume a density of the particle as 1.1 and an iron content of 50 per cent. Since 1.0×10^{-7} micromoles or 55.85×10^{-13} grams are needed per cell we have, where r is the radius of the particle,

$$55.85 \times 10^{-13} = \tfrac{4}{3}\pi r^3 \times 1.1 \times \tfrac{1}{2} \quad \text{or} \quad r = 2 \times 10^{-4}\ \text{cm.} = 2\mu.$$

From the above relationships it is noted that a particle of 4 μ radius would furnish enough iron for three divisions.

The use of iron content as a parameter in a nutrient index of a productivity equation now presents itself. However, unlike necessary ionic species, the absolute concentration of iron does not reflect its availability. A direct study is needed of the number of particulate iron particles and their size distribution. One possible approach to this problem has already been initiated in this laboratory. Sea water is filtered through a cellulose membrane filter which retains particles of 0.1 μ in diameter. Following the filtration the membrane is washed with distilled water and dried. The membrane is then cleared with polyoxyethylene sorbitan monolaurate which is saturated with potassium thiocynate and is acidified with 3 drops HCl per 100 ml. The typical red iron thiocyanate color is produced about any iron particle. By the use of this technique, a study of the size distribution of the iron particles in the euphotic zone of Pacific coastal waters will be made.

SUMMARY

1. With the use of radioactive iron it was found that the marine diatom *Asterionella japonica* utilized only particulate and/or colloidal iron as a growth nutrient, whereas ionically complexed ferric ion as the citrate, ascorbate, or artificial humate was not available for uptake.

2. A minimal content of iron per cell needed for further division was established as 10×10^{-8} micromoles per liter. The ratio of the minimal iron to the minimal phosphate agrees substantially with the iron-to-phosphate ratio in natural plankton samples.

LITERATURE CITED

Buch, Kurt, 1942. Determination of traces of Fe with alpha-alpha' bipyridyl. *Finska Kemistsamfundets Medd.*, **51**: 22–39.

Burk, D., Hans Lineweaver and Kenneth Horner, 1932. Iron in relation to the stimulation of growth by humic acid. *Soil Sci.*, **23**: 413–451.

Cooper, L. H. N., 1935. Iron in the sea and in marine plankton. *Proc. Roy. Soc. Lond.*, **118B**: 419–438.

Cooper, L. H. N., 1948. The distribution of iron in the waters of the western English Channel. *J. Mar. Biol. Assoc.*, **25**: 279–313.

Goldberg, E. D., T. Walker and A. Whisenand, 1951. Phosphate utilization by diatoms. *Biol. Bull.*, **101**: 274–284.

Gran, H. H., 1933. Studies on the biology and chemistry of the Gulf of Maine. II. Distribution of the phytoplankton in August, 1932. *Biol. Bull.*, **64**: 159–181.

Harvey, H. W., 1937a. The supply of iron to diatoms. *J. Mar. Biol. Assoc.*, **22**: 205–219.

Harvey, H. W., 1937b. Note of colloidal ferric hydroxide in sea water. *J. Mar. Biol. Assoc.*, **22**: 221–225.

Harvey, H. W., 1945. Recent advances in the chemistry and biology of sea water. Cambridge University Press.

Hopkins, E. F., 1930. Iron ion concentration in relation to growth and other biological processes. *Bot. Gaz.*, **89**: 209–240.

Jacobson, L., 1951. Maintenance of iron supply in nutrient solutions by a single addition of ferric potassium ethylenediamine tetra-acetate. *Plant Phys.*, **26**: 411–413.

Peltz, S. I., and E. V. Lynn, 1938. Decomposition of citric acid by ferric ion. *J. Amer. Pharm. Assoc.*, **28**: 774–775.

Riley, G. A., H. Stommel and D. F. Bumpus, 1949. Quantitative ecology of the plankton of the western north Atlantic. *Bull. Bingham Oceanogr. Coll.*, **12**: 1–169.

Rodhe, Wilhelm, 1948. Environmental requirements of fresh water plankton algae: Experimental studies in the ecology of phytoplankton. *Symb. Bot. Upsalienses*, **10**: 1–149.

Ross, J. F., and M. A. Chapin, 1942. The electrolytic separation of radioactive iron from the blood. *Rev. Sci. Instr.*, **13**: 77–80.